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# Evaluating UV-B Effects and EDU Protection in Soybean Leaves Using Fluorescence

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## ABSTRACT

**A growth-chamber experiment was conducted to evaluate whether ethylenediurea (EDU), a chemical shown to be protective against ozone pollution, could ameliorate foliar damage induced by ultraviolet-B (UV-B) radiation exposure in 'Roanoke' soybean (*Glycine max* L.), a UV-B–sensitive cultivar, and whether these effects could be discriminated using fluorescence (F) observations. The experiment had four treatment groups: control; biologically effective UV-B (18 kJ $m^{-2}$ $day^{-1}$); EDU (500 μmol $mol^{-1}$); and both UV-B and EDU (UV/EDU). Measurements included photosynthetic pigments, F image system (FIS) images of adaxial surfaces in four spectral regions (blue, green, red and far-red) and F emission spectra of the pigment extracts produced at two excitation wavelengths, 280 nm (280EX) and 380 nm (380EX). Several F ratios from 280EX, 380EX and the FIS images successfully separated the low UV *vs* high EDU group responses based on means alone, with intermediate values for controls and the combined UV/EDU groups. A UV-B/blue emission ratio, F315/F420 (280EX), was correlated with chlorophyll content (μg $cm^{-2}$)($R = 0.88$, $P < 0.001$), as was a ratio of emissions at two UV-A wavelengths: F330/F385 (280EX) ($R = 0.87$). These two 280EX ratios were also linearly correlated with emission ratios produced by 380EX, such as the far-red/green ratio, F730/F525 (380EX) ($R = 0.92$, $P < 0.001$), and clearly distinguished the UV-B and EDU groups separately, and which bracketed the similar intermediate responses of the UV/EDU and control groups. The FIS images additionally captured the following anatomical spatial patterns across the leaf surfaces: (1) emissions of UV-B–irradiated leaves were more uniform but lower in intensity than those of other groups; and (2) emissions of EDU-treated leaves exhibited the greatest variation in spatial patterns because veins had elevated blue F and leaf edges had enhanced red and far-red F. This experiment supports the hypothesis that EDU substantially ameliorated UV-B damage to foliage, a result that relied on the combined use of FIS images and emission spectra.**

## INTRODUCTION

Ultraviolet-B (UV-B, 280–320 nm) irradiation damage to vegetation is well documented (1). The most common responses to long-term UV-B exposure over a growing season are reduced photosynthetic pigment content and photosynthetic capacity, altered root-to-shoot ratios and altered foliage characteristics, which together lead to reduced leaf area, plant height and biomass. Protective mechanisms also develop (2–4), such as a thicker epidermis, accumulation of flavonoids (UV-B absorbing compounds) and hydroxycinnamic acids and a more lateral growth pattern. For short-term exposures, as in growth-chamber experiments, photosynthetic dysfunction may develop but changes in pigment content are inconsistent; a typical early protective response can be an increase in photosynthetic pigments (4–6). A means to ameliorate the potentially harmful exposures of spring and summer UV-B irradiation levels experienced by low- and mid-latitude crops could have economic benefits to producers. Ethylenediurea (EDU) is a compound successfully used in a soil drench on crops to ameliorate ozone damage (7–9). An earlier study found that soil uptake of EDU partially ameliorated UV-B exposures in cucumber, a sensitive crop (10). That study also examined the use of fluorescence (F) emission spectra and multiband F images in detecting foliage responses to UV-B and EDU. Chlorophyll fluorescence (ChlF) has been used for decades to evaluate photosynthetic function (11–14). F images (15–17) and spectra (18–24) have been used successfully to detect physiological status. F has also been used to investigate plant UV-B stress (10,25–28).

Given the protective role of EDU against UV-B–induced damage in our previous cucumber study (10), we extended our research to examine UV-B exposure and EDU uptake in a UV-B–sensitive soybean cultivar. In the current study, we con-

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*Abbreviations:* 280EX, fluorescence excitation wavelength at 280 nm; 380EX, fluorescence excitation wavelength at 380 nm; ChlF, chlorophyll fluorescence; DMSO, dimethyl sulfoxide; EDU, ethylenediurea; F, fluorescence; FIS, fluorescence imaging system; GLM, global linear model; HPS, high-pressure sodium; LPS, low-pressure sodium; MH, metal halide; PAR, photosynthetically active radiation; RFI, relative fluorescence intensity; UV-B, ultraviolet-B; $UV\text{-}B_{BE}$, biologically effective UV-B.

ducted a set of two experiments in sequence to evaluate the following: (1) whether EDU could provide protection against UV-B radiation; (2) whether UV-B and EDU effects could be discriminated using F spectral observations; (3) whether similar or different information was expressed by F spectra *vs* F images of leaves provided with EDU and UV-B exposure; and (4) whether the age of leaves influenced the F properties observed.

## MATERIALS AND METHODS

### Plant material and treatments

Two replicated factorial experiments (Exp. 1, Exp. 2) were conducted in July 1996 with four treatment groups: control (no UV, no EDU); EDU; UV-B; and UV-B and EDU together (UV/EDU). Five plants per treatment were included in each experiment (n = 40). The UV-B irradiation levels were equal in the two experiments. 'Roanoke' soybean (*Glycine max* L.) was used as experimental plant material because it was found previously to be sensitive to elevated UV-B radiation (Krizek 1992, unpublished results). Plants were grown in 12.7 cm diameter plastic pots containing 200 g of peat–vermiculite mix (Jiffy Mix, Jiffy Products of America, West Chicago, IL), fertilized daily with a complete nutrient solution, as described by Silvius *et al.* (29), and thinned to one plant per pot 4 days after germination.

At the time of full cotyledon expansion (after about 7 days), plants were given a soil drench of EDU at either 0 or 500 μmol $mol^{-1}$ on 15 July (Exp. 1) and 22 July (Exp. 2). Plants were then placed in a growth chamber for 2 weeks under the following environmental conditions: 27°C day/night temperature, 50% relative humidity and 350 μmol $mol^{-1}$ $CO_2$. The chamber contained an equal mix of 400W high-pressure sodium (HPS) and 400W metal halide (MH) lamps which provided 840 μmol $m^{-2}$ $s^{-1}$ photosynthetically active radiation (PAR, 400–700 nm) over a 12 h photoperiod (between 0730 and 1930 h), with 270 μmol $m^{-2}$ $s^{-1}$ provided for a 1 h transition period immediately before and after the photoperiod (0630–0730 h and 1930–2030 h).

Subsequently, the plants were transferred for UV treatment to a separate growth chamber containing 180W low-pressure sodium (LPS) lamps (Phillips North America, Bloomfield, NJ) to provide background PAR for 14 h (0630–2030 h) as well as supplemental UV irradiation as described below. The UV exposure chamber was maintained at the same temperature, relative humidity and $CO_2$ concentration as the HPS/MH growth chamber. The LPS chamber was divided into two compartments by a vertical sheet of UV-B–absorbing polyester film. UV-B 313 fluorescent lamps (Q-Panel Lab Products, Cleveland, OH) were mounted horizontally at ~0.5 m above the plants in both compartments. The lamps over the "no UV" compartment were wrapped with polyester (0.13 mm thick) to block UV-B irradiation, and those over the "UV" compartment were wrapped with cellulose diacetate (0.08 mm) to transmit UV-B radiation. The plants in these two compartments were exposed to either 0.2 or 18 kJ $m^{-2}$ $day^{-1}$ of biologically effective UV-B ($UV\text{-}B_{BE}$) radiation, normalized to unity for an 8 h period in the center of the 14 h photoperiod. The UV-B exposures were continued for 10 days. UV-B irradiance at plant level was monitored with a portable UV radiometer (Minimum Erythemal Dose, MED Meter, Solar Light Co., Philadelphia, PA), calibrated with a UV spectroradiometer (model 752, Optronics Laboratory, Inc., Orlando, FL), and adjustments were made to maintain constant exposure levels. Following UV-B irradiation, plants were returned to the HPS/MH growth chamber.

The treatments are summarized: (1) control plants (0.2 kJ $UV\text{-}B_{BE}$ $m^{-2}$ $day^{-1}$, no EDU); (2) UV-B–exposed plants (18 kJ $UV\text{-}B_{BE}$ $m^{-2}$ $day^{-1}$, no EDU); (3) EDU-exposed plants (0.2 kJ $UV\text{-}B_{BE}$ $m^{-2}$ $day^{-1}$, 500 μmol $mol^{-1}$ EDU); and (4) plants provided combined UV-B and EDU exposures (18 kJ $UV\text{-}B_{BE}$ $m^{-2}$ $day^{-1}$, 500 μmol $mol^{-1}$ EDU). Two experiments were conducted in sequence in 1996, each with 10 days of UV-B irradiation and EDU uptake (after a single EDU application).

### Measurements

After 10 days of UV-B irradiation and EDU uptake, measurements in both experiments were made of pigment content and spectral F emissions, on both the oldest (unifoliate) leaf and the youngest fully expanded trifoliate leaf per plant.

*Multispectral fluorescence images.* A custom F imaging system (FIS) was used to acquire steady-state F emission images of whole leaves or leaflets in four spectral bands (30). The band regions, with their center wavelengths and full widths at half-maximum were the blue (450, 25 nm), green (550, 25 nm), red (680, 10 nm) and far-red (740, 10 nm). Emissions in these bands were actively induced by a broadband (300–400 nm) UV excitation source consisting of four 15W long-wave UV-A lamps with peak output centered at 365 nm (UV-A fluorescent lamps, model EA-180/12, Spectroline Inc., Westbury, New York) filtered with Schott UG-1 glass to eliminate radiation >400 nm. The lamps were arranged at a 45° angle toward a central target area approximately 0.2 m above the sample surface to provide nearly uniform broadband illumination with an intensity of 0.33 mW $cm^{-2}$. The FIS consists of a thermoelectrically cooled digital camera and optics (Lynxx-2 charge coupled device [CCD] camera; Spectra Source Instruments, Westlake Village, CA), a motorized filter wheel (CVI Inc., Albuquerque, NM) and a desktop computer interface for data storage and instrument control. A horizontal surface painted nonfluorescent flat black served as the platform for leaf samples, situated ~0.5 m below the down-looking CCD camera.

Freshly excised leaves were placed on the platform with adaxial surfaces upward to be viewed by the CCD. For each image, four leaves (or central leaflets of trifoliate leaves) were placed in a fixed arrangement on the platform (*e.g.* replicate #2 of each treatment group), one per treatment arranged clockwise from the upper left corner in this order: control, UV, UV/EDU, EDU. Nonsaturated images were acquired in each of the four spectral bands for each replicated set. A simple threshold method was used to create a masked image of leaves in each spectral band, within which F means and standard deviations (SD) for masked regions of each of the individual leaves were determined using image processing software developed in MS Windows (Visual Basic V. 6.0, Microsoft Corp., Redmond, WA). Images were also produced for "regions of interest" to enhance the spatial F patterns across the adaxial lamina that resulted from localized treatment effects. Leaf surface regions expressing emissions similar to those of the control, UV and EDU groups were calculated and mapped as those regions exhibiting emissions within the ~99th percentile of the intensity histogram, per treatment mean. The percent coefficient of variation (CV) per treatment was computed ([SD ÷ mean] × 100) for FIS emissions of trifoliates from Exp. 2 to capture spatial variation across the leaf surface in each band.

*Photosynthetic pigments and UV-B–absorbing compounds.* Immediately following the FIS acquisitions, two leaf disks (1 cm diameter) were removed from the distal portion of these leaves for extraction of photosynthetic pigments (27). The disks were extracted in 4 mL dimethyl sulfoxide (DMSO) and kept for 24 h in the dark. Samples of the pigment extracts were placed in standard quartz cuvettes and analyzed in a computerized dual beam spectrophotometer (Lambda 3B, UV/VIS, Perkin-Elmer, Norwalk, CT). The absorption spectra were scanned at 1 nm resolution from 400 to 750 nm and used in equations described by Wellburn *et al.* (31) to calculate concentrations of photosynthetic pigments expressed on a per area basis (μg $cm^{-2}$), as also discussed by Barnes *et al.* (32).

An additional four leaf disks (1 cm diameter) were removed from the distal portion of each leaf for extraction of UV-B–absorbing compounds in ethanol acidified with glacial acetic acid (vol:vol, ethanol:acetic acid, 99:1). The disks were boiled gently for 10 min in a water bath at 80°C, and absorbances were read at 270, 300, and 330 nm (A270, A300, A330) using a UV-Visible Recording Spectrophotometer (UV-160A, Shimadzu, Columbia, MD) to assess relative concentrations of UV-absorbing compounds.

## CONCLUSIONS

This study demonstrates that UV-B and EDU effects on plants of a sensitive soybean cultivar can be remotely detected with F images of intact leaves, as well as by line spectral fluorescence of leaf extracts. These two stressors act differently on the plants, and their effects are manifested differently in the F characteristics. The F images we obtained are complex to interpret, but express a wealth of physiological, morphological, developmental and spatial information about leaf responses to environmental stresses. The localized within-leaf responses to the treatments shown in the F images enabled us to better elucidate the physiological effects of UV-B and EDU exposures.